REMARKS/ARGUMENTS

Applicants respectfully request reconsideration and allowance of this application in view of the amendments above and the following comments.

Claims 45, 47 and 48 have been amended to clarify that the promoter is a ***ubiquitous*** polymerase III-dependent promoter in accordance with the specification at page 8, lines 4-13. Applicants do not believe that the amendments to these claims introduce any new matter. An early notice to that effect is earnestly solicited.

Claims 45, 47 and 48 were rejected under 35 USC § 103(a) as being obvious over Lowe et al. ("Lowe"), US 2008/0226553, in view of Beach et al. ("Beach"), US 2004/0086884. In response, Applicants respectfully submit that the combination of Lowe in view of Beach does not make out a *prima facie* case of the obviousness of any of the rejected claims. Therefore, Applicants respectfully request that the Examiner reconsider and withdraw this rejection.

Applicants respectfully submit that a person having ordinary skill in the art, given the combination of Lowe and Beach, would not have had a reasonable expectation that a shRNA construct under the control of a ubiquitous RNA polymerase III (pol III) dependent promoter could mediate RNA interference *in vivo wh*en integrated into a RNA polymerase II (pol II) dependent locus.

Beach demonstrate that a luciferase specific shRNA under the control of the pol III dependent U6 promoter can mediate widespread gene silencing in cultured cell lines (ambiguously referred to as "*in vivo*" in this document). Random rather than targeted integration

of shRNA expression vectors is applied in all experiments presented and the resulting cell lines were not further analyzed concerning the integration site or the number of shRNA copies integrated into the genome. Usually, random integration of DNA vectors results in a concatameric array of multiple copies, whereas single copy integrations are unusual (Martin, *BioAssays 18:* 919-923 (1996)). In addition, the activity of a shRNA construct in a transgenic animal is not disclosed. Therefore, Beach does not teach anything about the requirements of the genomic environment to facilitate transgenic shRNA expression *in vivo*.

Lowe teaches a luciferase specific shRNA expression vector embedded in the hprt gene locus of the mouse genome such that the construct is operatively linked to the endogenous, pol II dependent hprt promoter. The activity of the modified hprt locus appeared to be limited, resulting in an incomplete suppression of luciferase activity *in vitro* (less than 5-fold relative to control cells, as shown in figure 24 of the document). Accordingly, previous attempts to achieve ubiquitous cDNA expression via targeted transgenesis at the hprt locus ***failed.*** Insertion of a lacZ gene under the control of the ubiquitous polyoma enhancer/HSV thymidine kinase promoter into the third exon of Hprt resulted in variable ß-galactosidase expression that was both orientation and cell-type dependent (Shaw-White, *Transgenic Res., 1:* 1-13 (1993)). Although transgenes under the control of the ubiquitous ß-actin gene promoter resulted in widespread expression when inserted into the Hprt locus, the level of transcripts varied strongly in different tissues (Bronson, *Proc. Natl. Acad. Sci. USA, 93(17):* 9067-72 (1996)). Unexpectedly, expression of these transgenes, but not of the endogenous Hprt gene appeared to be low or undetectable in kidney and liver (Bronson, *Proc. Natl. Acad. Sci. USA, 93(17):* 9067-72 (1996)). Hatada demonstrated that the HPRT locus suppresses the activity of both the tissue-specific haptoglobin gene promoter as well as the ubiquitous herpes simplex thymidine kinase promoter in several

tissues of mice (Hatada, *J. Biol., Chem., 274(2):* 948-55 (1999)). Likewise, a human eNOS promoter-LacZ reporter gene placed in the Hprt locus was found to be inactive in hepatic vessels that otherwise express the endogenous eNOS gene (Guillot, *Physiol. Genomics, 2:* 77-83 (2000).

None of the cited documents are instructive of the requirements of the genomic environment to facilitate expression of a shRNA construct driven by a heterologous, ubiquitous RNA polymerase III dependent promoter in a transgenic animal. Given that even pol II dependent promoters in the context of hprt exert a limited activity, persons skilled in the art would not have had a reasonable expectation that a ubiquitous pol III dependent shRNA construct could mediate RNA interference *in vivo* when integrated into a pol II dependent locus.

Thus, taken together, the combination of Lowe and Beach did not teach or suggest to persons skilled in the art that they should or could introduce a hRNA expression vector under the control of a ubiquitous, pol III dependent promoter into the hprt locus with a reasonable expectation of success. Absent such reasonable expectation of success, the instant claimed invention cannot be *prima facie* obvious over the combination of Lowe and Beach.

Moreover, as can be seen from the experimental portion of the instant application, the polymerase II locus in combination with a heterologous polymerase III-dependent locus provides for efficient repression of a target gene through expression of the appropriate shRNA construct (see Example 4). This showing is, thus, in view of the lack of a reasonable expectation of success in the prior art, in the nature of a showing of unexpected results. Although these data are not in declaration form, consistent with the rule that *all* evidence of nonobviousness must be considered when assessing patentability, the Examiner must consider data in the specification in

determining whether the claimed invention provides unexpected results. *In re Soni,* 34 USPQ2d 1684, 1687 (Fed. Cir. 1995).

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw this rejection. An early notice that this rejection has been reconsidered and withdrawn is earnestly solicited.

Applicants believe that the foregoing constitutes a bona fide response to all outstanding objections and rejections.

Applicants also believe that this application is in condition for immediate allowance. However, should any issue(s) of a minor nature remain, the Examiner is respectfully requested to telephone the undersigned at telephone number (212) 808-0700 so that the issue(s) might be promptly resolved.

Early and favorable action is earnestly solicited.

Respectfully submitted,
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